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The present invention provides DNA sequences encoding complementarity determining regions of variable domains of human anti-RhD antibodies and their use in the production of recombinant chimaeric antibody molecules.

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Monoclonal Antibodies

5        This invention relates to novel monoclonal anti-RhD antibodies prepared by recombinant DNA methods.

      The Rhesus blood group system is a major antigenic constituent of the human red blood cell membrane; of this group, the RhD antigen is of particular clinical  
10       importance in relation to isoimmune reactions. An Rh D- individual with anti-RhD who receives RhD+ blood is liable to suffer substantial red blood cell (RBC) destruction due to the RhD phenotype incompatibility, and thus blood of donors must routinely be classified as  
15       RhD+ or RhD-. Anti RhD monoclonal antibodies (antiD Mabs) are capable of providing blood-typing reagents of high specificity and reliability.

      The RhD antigen is also responsible for haemolytic disease of the newborn (HDN). This condition arises in  
20       newborn RhD+ infants of RhD- mothers previously sensitised to RhD antigen as a result of IgG anti-RhD antibodies crossing the placenta during pregnancy and causing foetal red blood cell (RBC) destruction. Sensitization of the RhD- mother to RhD antigen often  
25       occurs during the birth of an earlier RhD+ child due to some foetal RBCs entering the maternal circulation and being recognised as foreign by the maternal immune system. To reduce the incidence of HDN, it is routine practice in the United Kingdom and many other countries  
30       to give anti-RhD antibodies to RhD- mothers immediately after the birth of an RhD+ infant so that any RhD+ RBCs which may have entered the maternal circulation are rapidly removed.

      The search for the most effective anti D Mabs has  
35       proved to be extremely time consuming, involving the isolation of B-lymphocytes from humans immunised against RhD, usually Rh-ve mothers who have given birth to Rh+ve

children. Such lymphocytes are subjected to EBV treatment to provide an immortalised cell-line directly or the EBV-treated cells are hybridised with suitable mouse myeloma cells to provide a hydridoma: The cell-  
5 line or hybridoma may then be used to produce the anti-D Mab in the conventional way.

However, there are significant differences between anti-D Mabs in terms of their binding affinities for red cells, their ability to recognise D-variants such as D<sup>u</sup> and D<sup>vi</sup>, and their ability to destroy target cells by  
10 phagocytosis or cell-mediated lysis. It is desirable, therefore, to have available a method of combining the favourable parameters of different anti-D Mabs or, indeed of combining the most favourable features of  
15 selected anti-D Mabs with Mabs of quite different specificities which present particular advantages, in order to produce so-called chimaeric Mabs.

The concept of building chimaeric Mabs, has been described by Jones et al (Nature 321, 522-525 (1986))  
20 and Riechmann et al (Nature 332, 323-327 (1988)). Three dimensional studies have shown that immunoglobulins comprise essentially constant regions common to most Mabs and terminally situated variable domains associated with antigen binding.

It has been shown that the variable domains consist of two  $\beta$ -sheets joined by a disulphide bridge with their hydrophobic faces in contact. Sequence comparisons  
25 among heavy- and light-chain variable domains ( $V_H$  and  $V_L$  respectively) have revealed that each of these domains comprises three hypervariable domains or complementarity  
30 determining regions (CDRs) set in a framework of four relatively conserved regions, the framework regions (FRs). The CDRs are primarily responsible for the recognition of specific antigens. The structure of the  
35  $\beta$ -sheet framework is similar in different antibodies, as the packing together of  $V_L$  and  $V_H$  FRs is conserved and therefore the orientation of  $V_L$  with respect to  $V_H$  is

fixed.

Genes coding for a number of Mabs are now available and the sequences coding for the variable regions  $V_L$  and  $V_H$  have been determined. It is thus possible to replace the latter sequences by DNA coding for  $V_L$  and  $V_H$  from different Mabs and indeed to construct the latter by incorporating DNA coding for chosen CDRs into DNA coding for a standard set of FRs. It is thus possible to construct genes coding for chimeric anti-D Mabs having the CDRs from anti-D Mabs possessing particularly desirable specificities or other properties and framework and constant regions derived from Mabs having other desirable properties.

It is a prerequisite of such construction that the amino acid sequences of the CDR regions of the chosen anti-D Mabs and/or the genes coding for them, should be known. The specific CDR gene sequences can then be synthesised, conveniently by chemical synthesis of the appropriate oligonucleotides, and incorporated into DNA sequences coding for a standard set of FRs and the human (or other) constant region. Of course, the FRs may be identical with those of the Mab providing the constant region or, more conveniently, they may be a standard set of FRs which can be used generally in the synthesis of chimeric Mabs.

We have produced a number of anti-D Mabs of particular interest and have determined their amino acid sequences, thus making it possible for DNA sequences corresponding to their CDRs to be synthesised and incorporated into  $V_H$  and  $V_L$  sequences as described above. These may then be combined with DNA coding for the constant region to enable novel anti-D Mabs to be synthesised which may have lower, the same or higher binding ability.

Thus, according to one aspect we provide DNA sequences comprising oligonucleotides encoding CDR1, CDR2, and CDR3 regions of  $V_H$  and  $V_L$  domains of antibodies

against the human RhD antigen, and functional equivalents thereof. In particular, we have investigated and sequenced eleven Mabs, namely a) FOG-B, b) PAG-1, c) MAD-2, d) FOG-1, e) FOM-1, f) FOM-A, g) BRAD-3, h) JAC-10, i) GAD-2, J) REG-A, K) HAM-B, whose heavy and light chain sequences are represented in figures 2-14, of the accompanying drawings, and which have both varied and particularly useful binding specificities. The figures 2 and 3 show the nucleotide and amino acid sequences of the light chain variable domains of the Mabs FOG-B and PAG-1. Corresponding sequences for the heavy chain variable domains of these two Mabs are shown in figures 4 and 5, and sequences of the heavy chain variable domains of the Mabs MAD-2, FOG-1, FOM-A, BRAD-3, JAC-10, GAD-2, REG-A and HAM-B are shown in figures 6-14.

Synthetic genes, for both heavy and light chains may be created by combining selected CDR 1, 2, and 3 regions, which may be selected from different antibody molecules having varied binding specificities.

Thus according to a further aspect, we provide DNA molecules coding for the heavy or light chain fragments of a monoclonal antibody or fragment thereof comprising CDR1, CDR2 and CDR3 encoding oligonucleotides from antibodies FOG-B, PAG-1, MAD-2, FOG-1, FOM-1, FOM-A, BRAD-3, JAC-10, GAD-2, REG-A and HAM-B as illustrated in figures 2-14.

In order to create functional genes, such oligonucleotides must be incorporated into a backbone sequence such that when expressed, functional proteins result.

Thus according to a further aspect, we provide DNA molecules comprising a gene coding for the framework regions of a human antibody light or heavy chain having inserted therein in the correct CDR region, oligonucleotides encoding CDR1, CDR2 and CDR3 regions according to the present invention.

In the synthesis of a chimeric Mab in accordance with the invention, single stranded DNA coding for the  $V_H$  region of a chosen Mab (not necessarily an anti-D Mab) is incorporated in single stranded form into a vector capable of producing single stranded DNA, such as the M13 bacteriophage. Fig. 1 shows diagrammatically the structure of a single stranded  $V_H$  DNA including framework regions FR1 to FR4 with complementarity determining regions CDR1 to CDR3 of a Mab. These steps can be accomplished by conventional techniques such as those described in Riechmann et al (Nature, 332, 323-327, (1988)).

Three oligonucleotides may then be prepared corresponding to the CDR regions of the chosen anti-D Mab variable domain, eg the  $V_H$  region of FOG-B as shown in Fig. 4, and will include several nucleotides on either side of each CDR region to permit hybridisation with the framework regions FR1 to FR4 (see figure 1). The sequences of the latter will normally be substantially homologous with those of the anti-D Mab (e.g. FOG-B) but since the oligonucleotides will normally be synthesised chemically, hybridisation may be ensured by matching the overlapping nucleotides exactly to the FRs 1 to 4. It may also be beneficial to modify the oligonucleotides to express the CDRs more efficiently in the eventual host cells.

The three oligonucleotides, shown in Fig 1 as oligo 1 to oligo 3, may then be annealed to a single stranded  $V_H$  DNA in the M13 vector and used as primers to synthesise second strand DNA containing the anti-D  $V_H$  CDR sequences. This may be achieved conventionally using a suitable polymerase. Since the antibody specificity is determined solely by the three CDR regions, the actual  $V_H$  gene chosen for the framework template is immaterial. All that is required is that there is sufficient homology of the three chosen oligonucleotides with the template. This is ensured by appropriate design of the

terminal nucleotides of the synthetic oligonucleotide primers. Thus the second strand may contain sequences from substantially any human antibody heavy chain gene, so long as the resulting expressed protein possesses the desired binding parameters.

The double stranded M13 vector may then be used to transform a suitable host microorganism e.g. a conventional E. coli and one or more clones selected which contain the required anti-D  $V_H$  specificity. The correct clone may be identified by DNA sequencing.

The corresponding  $V_L$  DNA (e.g. for FOG-B) may be prepared in the same way.

The DNA coding for the  $V_H$  and  $V_L$  regions may then be excised from the above vectors and introduced into other vectors.

According to a further aspect, we provide DNA molecules being synthetic genes for chimaeric antibody, heavy or light chains when incorporated into vectors capable of expressing such antibody chains. Preferred vectors include mammalian expression vectors, such as pSV2gpt (heavy chains) and pSV2neo (light chains) containing DNA coding for the human constant region. Such vectors are readily available from a number of laboratories, or can readily be prepared by incorporating DNA coding for human constant region into known mammalian vectors.

The expression vectors so constructed may then be co-transfected into an appropriate cell-line e.g. a non-secreting IgG myeloma, for large scale production.

Thus according to a yet further aspect, the present invention provides each of the CDR polypeptides of the Mabs FOG-B, PAG-1, MAD-2, FOG-1, FOM-1, FOM-A, BRAD-3, JAC-10, GAD-2, REG-A and HAM-B shown in Figs. 2-14 of the accompanying drawings in single stranded or double stranded form in the absence of the constant and or framework regions of said Mabs.

According to a yet further aspect, the invention



provides chimaeric antibody heavy and light chains of the variable domains comprising CDR polypeptide sequences of the present invention.

- 5 Knowledge of the antibody sequences according to the invention enables new chimaeric anti-D antibody molecules to be prepared, having appropriately designed binding specificities. These antibodies may be used for both therapy and diagnosis using presently known techniques.

- 10 According to a yet further aspect, we provide anti-RhD reagents comprising at least one antibody molecule according to the invention.

- 15 According to a still yet further aspect, we provide pharmaceutical compositions for use in passive immunisation to prevent haemolytic disease of the newborn comprising an antibody of the present invention together with at least one pharmacologically acceptable carrier or diluent.

- 20 A sterile solution of such an antibody for human injection may be formulated in any physiologically acceptable aqueous medium, for example isotonic phosphate buffered saline or serum. Alternatively, the antibody may be supplied in a freeze-dried formulation ready for reconstitution prior to use.

EXAMPLE(1) Construction of Chimaeric Antibody Genes

5        Three oligonucleotide primers are synthesised using  
an Applied Biosystems machine according to the  
manufacturer's instructions and purified on an 8 M  
Urea/polyacrylamide gel (Sanger & Coulson, Febs Lett.,  
87, 107-110, 1978). The primers are designed to  
10        comprise in their central regions sequences  
complementary to the CDR1, CDR2 and CDR3 regions of the  
anti-RhD antibody PAG-1 heavy chain gene, as identified  
according to the criteria described by Kabat et al.  
(Sequences of Proteins of Immunological Interest, US  
15        Department of Health and Social Services, 1987).

The central sequences are flanked at both their 5'  
and 3' termini by sequences of 10 nucleotides which  
hybridise to the termini of the corresponding framework  
region sequences adjacent to the CDR sequence of the  
20        heavy chain antibody gene NEWM (Poljack et al.,  
Biochemistry 16, 3412-3420, 1977). The primers are then  
hybridised to the derived NEWM single stranded DNA heavy  
chain sequence in the M13 bacteriophage and the  
complementary strand of the heavy chain variable region  
25        extended using DNA polymerase (Neuberger et al., Nature  
314, 268-270 (1985), Jones et al., Nature 321, 522-5  
(1986)). The M13 vector also contains an appropriate  
arrangement for ultimate expression, i.e. a leader  
sequence, and unique HindIII and BamHI restriction  
30        sites.

A similar construct is prepared from  
oligonucleotide primers homologous to the CDR regions of  
the PAG-1 anti-RhD antibody light chain genes, and  
utilising the M13 vector in which V<sub>L</sub> and J<sub>L</sub> regions of  
35        the antibody gene PAV1 (Sun et al., Nucleic Acids  
Research 13, 4921-4934, 1985) are cloned.

(2) Expression of Antibody Polypeptides

The cloned genes for the V<sub>H</sub> domains are excised  
5 using HindIII and BamHI and cloned into pSV2gpt  
(Mulligan and Berg, PNAS 78, 2072-6, 1981). The cloned  
light chain genes are similarly excised and cloned into  
pSV2neo (Southern and Berg, J. Molec. Appl. Genetics 1  
327-381, 1981). Sequences encoding IgG1 constant  
10 regions are then inserted into the vectors (Riechmann et  
al., Nature 312, 323-7, (1988). Both vectors are then  
transfected by electroporation (Potter et al., PNAS 81,  
7161-3, 1984) into the rat myeloma cell line  
YO (YB2/3.0 AG, 20) (Galfre and Milstein, Methods in  
15 Enzymology 73, 1-46, 1981) for antibody production.

CLAIMS

1. A DNA sequence comprising an oligonucleotide  
encoding a CDR1, CDR2 and/or CDR3 region of a  $V_H$  or  
5  $V_L$  domain of an antibody against the human RhD  
antigen, and functional equivalents thereof.

2. A DNA sequence as claimed in claim 1 encoding the  
CDR1 region of a  $V_H$  domain selected from:

10

AGTGGTGGTCTCTACTGGGGC;

AGTTCCTACTGGAGC;

GGTTACTACTGGAGC;

GTTTACTACTGGACC;

15

GGTTACTACTGGAAC;

GGTTACTACTGGAGC;

AGCTATGGCATGCAC;

AGTTACTGGATGCAC;

AGCTATGGCATGCAC;

20

AATTATGGCATGCAC; and

AGCTATGGCATGCAC,

optionally with extended terminal regions.

25 3. A DNA sequence as claimed in claim 1 encoding the  
CDR2 region of a  $V_H$  domain selected from:

AGTATATTTTATAGTGGGAGCACCTACTACAATCCCTCCCTCAAGAGC;

TATATCTATTACAGTGGGAGCACCAACTACAACCCCTCCCTCAGGAGT;

30

GAAATCAATCATAGTGAAGGACCAACTACAACCCGTCCCTCAAGACT;

GAAATCAATCATAGTGGAGGCGCAACTACAATCCGTCCCTCAAGAGT;

GAAATCATTCATAGTGAAGCACCAACTACAACCCGTCCCTCAAGAGT;

GAAATCAGTCGTCGTGGAAGCACCAACTACAACCCGTCCCTCAAGAGT;

CTTATATGGTATGATGGAAGTAATAAAGAATATGCAGACTTCGTGAAG

35

GGC;

CGTATTAATAGTTATGGAATTAGCACAAAGTTACGCGAACTCCGTGAAG

GGC;

GTGATATGGTATGATGGAAGTAATAAGTACTATGCAGAGTCCGTGAAG  
GGC;  
GTTATATGGTATGATGGAAGTAATAAAACTATGCAGACTCCGTGAAG  
GGC; and  
5 GTTATTTGGTATGATGGAAGTAATAAATACTATGCAGACTCCGTGAAG  
GGC,

optionally with extended terminal regions.

- 10 4. A DNA sequence as claimed in claim 1 encoding the  
CDR3 region of a  $V_H$  domain selected from:

CCAGGCTATGGCGACACCTCGGTACGGAAGAGGGTTTGAATATGGAC  
CTC;  
15 GTTTTGGTTTCCCGTACCATTTCACAGTACTCCTATTACATGGACGTC;  
GTTTTGGTTTCCCGTACGATTTCACAGTACTCCTATTACATGGACGTC;  
CTGTGGCTCGATGGACATGGGTACAAGTTTGACTAC;  
GGCCGGTCCCGTTATAGTGGTTACGGCTTCTACTCCGGCATGGACGTC;  
GGCTTAGAACGTCCGATTAGGAACCAGCTGCTAAACCGTCTCGGTTAC  
20 TACATGGACGTC;  
GCCTTGGACTACATCTCCTTGGATTACGGTATGGACGTC;  
GATAGTCCCAAATGAGGGCTGGAAGTATGTTTCGCTACTACTACATG  
GACGTC;  
GGAGAGCGCATAGCAGCTCGTCTCTTGTCGGGCGGGTACGGTATGGAC  
25 GTC;  
GTCGTTAGCAGCAACCGGTACTCTTAAGCTACTATTATTACTACATGGAC  
GTC;  
GAACGTACTACGATGTCTGGAGTGATCATTCTCGCCGSTATTTTGAC  
TAC; and  
30 GAAGTTACTATGGTTCGGGGAGTTAGGCGTTACTACGGTATGGACGTC,

optionally with extended terminal regions.

5. A DNA sequence as claimed in claim 1 encoding the  
35 CDR1 region of a  $V_L$  domain selected from:

TCCGGAACCAGCTCCAACATTGGGAATAATTATGTATCC;

12

GGGGGAAACAACATTGGGCGTAAAAGTGTGCAC; and  
GGGGGAAACAACATTGGACGTAAAAGTGTGCAC,

optionally with extended terminal regions.

5

6. A DNA sequence as claimed in claim 1 encoding the CDR2 region of a  $V_L$  domain selected from:

GACAATAATAAGCGACCCTCA;  
GGTGCTAGCGAGCGGCCCTCA; and  
GGTGCTAGCGACCGGCCCTCA,

10

optionally with extended terminal regions.

15

7. A DNA sequence as claimed in claim 1 encoding the CDR3 region of a  $V_L$  domain selected from:

GCAACATGGGATAGCAGCCTGAGTGCTGTGGTG; and  
CAGGTGTGGGATAGTAGTAGTGCTCATCCGGGGGTGGTA,

20

optionally with extended terminal regions.

8. A DNA sequence as claimed in any one of claims 2 to 7 wherein the said extended terminal regions hybridise with the terminal sequences of the framework regions of a human antibody heavy or light chain gene flanking the CDR region.

25

9. A DNA molecule for the synthesis of a synthetic gene coding for the heavy chain fragment of a monoclonal antibody or fragment thereof comprising a CDR1 encoding oligonucleotide as claimed in claim 2, a CDR2 encoding oligonucleotide as claim in claim 3 and a CDR3 oligonucleotide as claimed in claim 4.

30

35

10. A DNA molecule for the synthesis of a synthetic gene coding for the light chain fragment of a monoclonal antibody or fragment thereof comprising a CDR1 encoding oligonucleotide as claimed in claim 5, a CDR2 encoding oligonucleotide as claimed in claim 6 and a CDR3 oligonucleotide as claimed in claim 7.
11. A DNA molecule comprising a gene coding for the framework region of a human antibody light or heavy chain having inserted therein for a heavy chain in the CDR1 position an oligonucleotide as claimed in claim 2, in the CDR2 position, an oligonucleotide as claimed in claim 3 and in the CDR3 position, an oligonucleotide as claimed in claim 4.
12. A DNA molecule comprising a gene coding for the framework region of a human antibody light or heavy chain having inserted therein for a light chain in the CDR1 position an oligonucleotide as claimed in claim 5, in the CDR2 position an oligonucleotide as claimed in claim 6 and in the CDR3 position an oligonucleotide as claimed in claim 7.
13. A DNA molecule as claimed in claim 11 or claim 12 when incorporated in a vector capable of expressing the said antibody heavy or light chain.
14. An expression vector as claimed in claim 13 which is replicable in mammalian cells.
15. A polypeptide sequence encoded by a CDR nucleotide sequence as claimed in any one of claims 2 to 7, and functional equivalents thereof.
16. A chimaeric antibody  $V_H$  or  $V_L$  chain or fragment thereof encoded by a DNA sequence as claimed

14

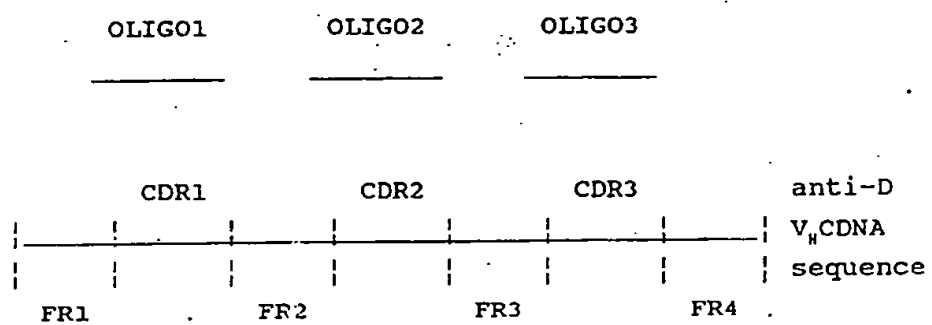
respectively in claim 11 or claim 12.

- 5 17. A chimaeric antibody molecule against the RhD antigen wherein the variable regions of the heavy and light chains comprise polypeptide sequences as claimed in claim 15.
- 10 18. An anti-RhD reagent comprising at least one antibody molecule as claimed in claim 17.
- 15 19. A pharmaceutical composition for use in passive immunisation to prevent haemolytic disease of the newborn comprising an antibody as claimed in claim 17 together with at least one pharmacologically acceptable carrier or diluent.
- 20 20. A method of Rh-typing wherein an antibody as claimed in claim 17 is employed.



FIG. 1

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2/14  
FOG-B VL SEQUENCE

```

1  CAGTCTGTGTTGACGCAATCCCTGCTGTCICIGLECCCCAGGACAGAGGTCACCATC  60
   U S V L T G P P S V S A A P G Q K V T I

61  TCCTGCTCCGGACCAAGCTCCACATGGGGAATATTAATGATATCTGGTATCAGCAGCTC  120
   S C S G T S N I G M N Y V S W Y Q D L
   <-----CDR1----->

121 CCAGGAACAGCCCCCAACTCCTCATTTATGACAATAATAGCGACCCICAGGGATTCTCT  180
   P G T A P K L L I Y D N N K R P S G I P
   <-----CDR2----->

181 GACCGATTCTCTGGCTCCAAAGTCIGGCACGTCAGGCCACCCCTGGGCATCACCGGACTCCGG  240
   D R F S G S K S G T S A T L G I T G L R

241 ACTGGGGACGAGGCCGATTATTACTGCGCAACATGGGATAGCAGCCCTGAGTGTGTGGTG  300
   T G D E A D Y Y C A T W D S S L S A V V
   <-----CDR3----->

301 TTGGGCGGAGGACCAAGCTGACCCGTCCTAAGT  333
   F G G G T K L T V L S
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PAG-1 VL SEQUENCE

1 TCCTATGTGCTGACTCAGCCACCCCTCGGTGTCAGTGGCCCCCAGACAGACGGCCAGGATT 60  
S Y V L T O P P S V S V A P G D T A R I

61 ACCTGTGGGGGAACACACATTGGACGTAAAGTGTGCACTGGTACCAAGCAGAAECCAGGEC 120  
T C G G N N I G R K S V H W Y Q O K P G  
<-----CDR1----->

121 CAGGCCCCCTGTGCTGGTCTGTATGGTGTAGCGACCGGCCCTCAGGGGATCCCTGAGCGA 180  
O A P V L V V Y G A S D R P S G I P E R  
<-----CDR2----->

181 TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCCTGACCATCAGCAGGGTCCGACGCCGGG 240  
F S G S N S G N T A T L T I S R V A A G

241 GATGAGGCCGACTATTACTGTGAGGTGTGGGATAGTAGTAGTCTCATCCGGGGGGTGGTA 300  
D E A D Y Y C Q V W D S S A H P G V V  
<-----CDR3----->

301 TTGGGCGGAGGGACCAAGCTGACCGTCCCTAGGT 333  
F G G G T K L T V L G

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FOG-B VH SEQUENCE

1 CAGCTGCGGCTGCAGGAGTCCGGGCCAGGACTGGTGAGCCCTCGGAGACCCCTGTCCTC  
G L R L O E S G P G L V K P S E T L S L 60

61 ACCTGCAGTGTCTCTGCTGGTGGCTCCGTCAGCAGTGGTGGTCTCTACTGGGGCTGGGTCCGC  
T C S V S G G S V S S G G L Y W G W V R 120  
-----CDR1----->

121 CAGCCCCCAAGGAGGCTCGAATGGATTGGCAGTATATTTTATAGTGGGAGCACCTAC  
Q P P G K G L E W I G S I F Y S G S T Y 180  
-----CDR2----->

181 TACATCCCTCCCTCAGAGCCGAGTCACCATATCCGTAGACACGTTGAAGAATAACTTC  
Y N F S L K S R V T I S V D T L K N N F 240  
----->

241 TCCCTGAAGCTGAGTTCTGTGACCCGCGCAGACACGGCTGTTTATTACTGTACGAGACCA  
S L K L S S V T A A D T A V Y C T R P 300  
----->

301 GGCTATGGCGACACCTCGGTACGGAGAGGGTTTGGAATATGGACCTCTGCGGGCCCAAGGG  
G Y G D T S V R K R V W N H D L W G D G 360  
-----CDR3----->

361 ACCACGGTCACCGTCTCCTCG 381  
T T V T V S S

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PAG-1 VH SEQUENCE

1 CAGGTGCAGCTGCAGGAGTCCGGGCCCAAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCGTC  
Q V D L D E S G P G L V K P S E T L S V 60

61 ACCTGCACCTGTCTCTGGTGGCTCCGTCAGTAGTTCCTACTGGAGCTGGATCCGGCAGGCC  
T C T V S G G S V S S Y W S W I R O P 120  
←-----CDR1-----→

121 CCAGGGAAGGACCGGAGTGGATTGGGTATATCTATTACAGTGGGAGCACCACACTACAAC  
P G K G P E W I G Y I Y Y S G S T N Y N 180  
←-----CDR2-----→

181 CCCCTCCCTCAGGAGTCGAGTCACCATATCAGTAGACACGTCACAGAACCCAGTTCTCCCTG  
P S L R S R V T I S V D T S K N O F S L 240  
←-----→

241 AAGCTGGGCTCTGTGACCGCTGCGGACACGGCCGTGTATTACTGTGCGAGAGTTTGGTT  
K L G S V T A A D T A V Y Y C A R V L V 300  
←-----→

301 TCCCGTACGATTTCACAGTACTCCTATTACATGGACGCTGCGGACCAAGGGACCCAGGTC  
S R T I S O Y S Y M D V W G K G T T V 360  
←-----CDR3-----→

361 ACCGTGTCCTCA 372  
T V S S

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MAD-2 VH SEQUENCE

1 -----PCR primer-----CGCAGGACTGTTGAAGCCTTCGGAGACCCCTGTCCCTC 60  
... . . . . A G L L K F S E T L S L

61 ACCTGCGCTGCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCGCCAGCCT 120  
T C A V Y G G S F S G Y Y W S W I R Q P  
<-----CDR1----->

121 CCAGGGAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAGGACCACTACAAAC 180  
P G K G L E W I G E I N H S G R T N Y N  
<-----CDR2----->

181 CCGTCCCTCAAGACTCGAGTCACCATATCAGTAGACACGTCACAGAACCAAGTTCTCCCTG 240  
P S L K T R V T I S V D T S K N Q F S L  
----->

241 AAGCTGAGTTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGACTGTGGCTC 300  
K L S S V T A A D T A V Y Y C A R L W L  
<----->

301 GATGGACATGGGTACAAAGTTTGACTACTGGGGCCAGGGAGACCCCT---PCR primer--- 360  
D G H G Y K F D Y W G O G T L . . . . .  
-----CDR3----->

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FOG-1 VH SEQUENCE

1 CAGGTGCATCTACAGCAGTGGGGCACAGGGCTGTGAGAGCCCTCGGAGACCCCTGTCCCTC 60  
O V H L O O W G T G L L K F S E T L S L

61 ACCTGGGCTGTCCATGGTGGTCCCTTCAATGTTTACTACTGGACCTGGATCCGCGCAGCC 120  
T C A V H G G S F N V Y Y W T W I R O F  
<-----CDR1----->

121 CCAGGAAAGGCGCTGGAGTGGATTGGGGAATCAATCATAGTGGAGGGCGCCAACTACAA 180  
P G K A L E W I G E I N H S G G A N Y N  
<-----CDR2----->

181 CCGTCCCTCAAGAGTGGAGTCACCATGTGAGCAGACACGTCGAGAACCCAGTTCTCCCTG 240  
P S L K S R V T M S A D T S K N O F S L  
----->

241 AAATGACCTCTGTGACCGCGCGGACACGGCTGTGTTTATTGTCGAGAGGGCCGGTCC 300  
K L T S V T A A D T A V F Y C A R G R S  
<-----CDR3----->

301 CGTTATAGTGGTTACGGCTTCTACTCCGGCATGGACGTCCTGGGGCCCGAGGGACCCAGGTC 360  
R Y S G Y G F Y S G M D V W G P G T T V  
-----CDR3----->

361 ACCGTCCTCTCA 372  
T V S S

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## FOM-1 VH SEQUENCE

1 CAGGTGCAGCTACAGCAGTGGGGGCGAGGACTGTTGAGGCTTCGGAGACCCIGTCCCTC 60  
Q V O L O O W G A G L L K P S E T L S L

61 ACCTGGGCTGTCTATGGTGGGTCCCTTCAGTGGTACTGGAACCTGGATCCGCCAGCCC 120  
T C A V Y G G S F S G Y Y W M I R O P  
<-----CDR1----->

121 CCAGGGGAAGGGGCTGGAGTGGATTGGGGAAATCATTCATAGTGGAGCACCAACTACAAC 180  
P G K G L E W I G E I I H S G S T N Y N  
<-----CDR2----->

181 CCGTCCCTCAAGAGTGGAGTCACCATGTCTAGTAGACACGTCACAGAACCCAGTTCCTCCCTG 240  
P S L K S R V T M S V D T S K N Q F S L  
<----->

241 AAGCTGAGCTCTGTGACCGCGGGGACACGGCTGTGTATTACTGTGGAGAGGCTTAGAA 300  
K L S S V T A A D T A V Y Y C A R G L E  
<----->

301 CGTCCGATTAGGGAACCAAGCTGCTAAACCGTCTCGGTTACTACATGGACGGTCTGGGGCAAA 360  
R P I R N O L L N R L G Y Y M D V W G K  
<-----CDR3----->

361 GGGACCAAGGTCACCGTCTCCTCA 384  
G T T V T V S S

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FOM-A VH SEQUENCE

1 CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCCTGTCCTC 50  
O V O L O O W G A G L L K F S E T L S L

61 ACCTGGGCTGTCATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCGGCCAGCCC 120  
T C A V Y G G S F S G Y Y W S W I R O P  
<-----CDR1----->

121 CCAGGGGAAGGGGCTGGAGTGGATTGGGGAAATCAGTCGTGGTGGAGCACCACACTACAAC 180  
P G K G L E W I G E I S R R G S T N Y N  
<-----CDR2----->

181 CCGTCCCTCAAGAGTCGAGTCGCCATATCAGTAGACACGTCACAGACCAAGTTCCTCCCTG 240  
P S L K S R V A I S V D T S K N O F S L  
<----->

241 AAGGTGAGGTCTGTGACCGCGGACACGGCTGTGTATTACTGTGCGAGAGCCCTTGGAC 300  
K V R S V T A A A D T A V Y C A R A L D  
<----->

301 TACAICCTCTGGATTACGGTATGGACGTCTGGGGCCCAAGGGACCAACGGTCACGGTCTCC 360  
Y I S L D Y G M D V W G O G T T T V T V S  
<-----CDR3----->

361 TCA 363  
S

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10/14  
BRAD-3 VH SEQUENCE

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1  -----PCR primer-----GGGAGGCGTGGTCCAGCCTGGGAGGTTCCCTGAGACTC      60
   . . . . . G G V V O P G R F L R L

61  TCCTGTGACGGCTCTGGATTACCTTCAGTAGCTATGGCATGGCACTGGGTCCGCCAGGCT      120
   S C A A S G F T F S S Y G H H W V R O A
   <-----CDR1----->

121 CCAGGCAAGGGGCTGGAGTGGGTGGCACTTATATGGTATGATGGAAGTAATAAGAAJAT      180
   P G K G L E W V A L I W Y D G S N K E Y
   <-----CDR2----->

181 GCAGACTTCGTGAAGGGCCGATTCCACCATCTCCAGAGACAATTCCAAGAATACACTGTAT      240
   A D F V K G R F T I S R D N S K N T L Y
   ----->

241 CTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACGTGCGACAGATAGT      300
   L O M N S L R A E D T A V Y Y C A T D S
   <----->

301 CCCAAATGAGGGCTGGAAGTATGTTTCCTACTACTACATGACGTCTGGGGCAAGGG      360
   P K N R A G S M F R Y Y M D V W G K G
   -----CDR3----->

361 ACCAC---PCR primer--- 381
   T . . . .

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11114  
JAC-10 VH SEQUENCE

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1  -----PCR primer-----GGGAGGGCTTAGATCAAGCCAGGGGGTCCCTGAGACTC      60
   . . . . . G G L V Q P G G S L R L

61  TCCGTGACAGCCCTCTGGATTACACCTTCAGTAGTTACTGGATGCACCTGGGTCCGCCAAGCT      120
   S C A A S G F T F S S Y W M H W V R O A
   <-----CDR1----->

121 CCAGGGAGAGGGGCTGGTGTGGGCTCAGCTATTAGTTATGGGAATTAGCACAAGTTAC      180
   P G K G L V W V S R I N S Y G I S T S Y
   <-----CDR2----->

181 GCGAAGCTCCGTGAAGGGCCGATTACCATCTCTCCAGAGACACGCCCAAGAACACGCTGTAT      240
   A N S V K G R F T I S R D N A K N T L Y
   ----->

241 CTGCAATGACACACTCTGAGAGCCGGGACACAGGCTGTATTACTGTGCAAGAGGAGAG      300
   L O M N T L R A E D T A V Y Y C A R G E
   <-----CDR3----->

301 CGCATAGCAGCTCTCTCTTGTGGGGGGGTACGGTATGGACGCTCTGGGGCCCAAGGGACC      360
   R I A A R L L S G G Y G M D V W G O G T
   ----->

361 AC-----PCR primer----- 378
   . . . . .

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GAD-2 VH SEQUENCE

```

1  -----PCR primer-----GGGAGGCGTGGTCCAGCCTGGGAGGTCCTGAGACTC   60
   . . . . . G G V V Q P G R S L R L

61  TCCTGTGCAGCGCTCTGGATTACCCITTAGTAGCTATGGCATGCACTGGGTCCGCCAAGGCT   120
   S C A A S G F T F S S Y G N H W V R O A
   <-----CDR1----->

121 CCAGGCAAGGGGCTGGAGTGGGTGGCAGTGATATGGTATGATGGAGTAATAAGTACTAT   180
   F G K G L E W V A V I W Y D G S N K Y Y
   <-----CDR2----->

181 GCAGAGTCCGTGAAGGGCCGATTCAACATCTCCAGGCGGCGATTCACCAAGAACACGCTGTAT   240
   A E S V K G R F T I S R D N S K N T L Y
   <----->

241 CTGCAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCGTT   300
   L O M N S L R A E D T A V Y Y C A R V V
   <----->

301 AGCAGCAACCGGTACTCTCTAAGCTACTATTATTACTACATGGACGCTCTGGGGCAAGGG   360
   S S N R Y S L S Y Y Y Y M D V W G K G
   <-----CDR3----->

361 ACCAC-----PCR primer----- 381
   T . . . . .

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13/14  
REG-A VH SEQUENCE

```

1  -----PCR primer-----GGGAGGCGTGGTCCAGCCCTGGGAGGTCCCTGAGACTC   60
   . . . . . G G V V Q P G R S L R L

61  TCCTGTGCAGCGTCTGGATTACCTTCAATAATTATGGCATGCACTGGGTCCGCCAGGCT   120
   S C A A S G F T F N N Y G M H W V R Q A
   <-----CDR1----->

121 CCAGGCCAAGGGGCTGGAGTGGGTGGCAGTTATATGTTATGATGGAAGTAATAAAACTAT   180
   F G K G L E W V A V I W Y D G S N K N Y
   <-----CDR2----->

181 GCAGACTCCGTGAAGGGCGGATTACCATCTCCAGAGACAAATCCAGAGACGCTGTAT   240
   A D S V K G R F T I S R D N S K N T L Y
   ----->

241 CTGCAATGAACAGCCCTGAGAGGCCGAGGACAGGCTGTGTATTACTGTGCGAGAGAACGT   300
   L O M N S L R A E D T A V Y Y C A R E R
   <----->

301 ACTACGATGTCTGGAGTGATCATTCCTCGCCGGTATTTTGACTACTGGGGCCAGGGGAACC   360
   T T M S G V I I P R R Y F D Y W G Q G T
   -----CDR3----->

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361 CG----PCR primer----- 378

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HAM-B VH SEQUENCE

```

1  -----PCR primer-----GGGCGCGTGGTCCAGCCCTGGGAGGTCCTGAGACTC      60
   . . . . . G G V V Q P G R S L R L
61  TCCTGTGCAGCGTCTGGATTACACCTTCAGTACCTATGSCATGCACTGGGTCCGCCAGGCT      120
   S C A A S G F I F S S Y G M H W V R O A
   <-----CDR1----->
121 CCAGGCAGGGGCTGGAGTGGGTGCGAGTTATTGGTATGATGGAAGTAATAATACTAT      180
   P G K G L E W V A V I W Y D G S N K Y Y
   <-----CDR2----->
181 GCAGACTCCGTGAAGGGCCGATTCCACCATCTCCAGAGACAAATCCAGAGACACGCTGTAT      240
   A D S V K G R F T I S R D N S K N T L Y
   ----->
241 CTGCAAAATGAACAGCCTGAGAGCGGAGACACGGCTGTGTATTACTGTGCGAGAGAAGTT      300
   L O M N S L R A E D T A V Y Y C A R E V
   <----->
301 ACTATGGTTCGGGGAGTTAGGCGTTACTACGGGTATGGACGTCTGCGGGCCCGAGGGACCAC-      360
   T M V R G V R R Y Y G N D V W G P G T
   -----CDR3----->
361 --PCR primer-- 375
   . . . . .

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 90/01964

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC C 12 N 15/13		
IPC <sup>5</sup> : C 07 K 15/28, C 12 P 21/08, A 61 K 39/395, G 01 N 33/80		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
IPC <sup>5</sup>	C 12 N, C 12 P, C 07 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> *		
Category *	Citation of Document, ** with Indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	GB, A, 2189506 (CENTRAL BLOOD LABORATORIES AUTHORITY) 28 October 1987 see the whole document	15-20
Y	---	1-14
Y	EP, A, 0239400 (G.P. WINTER) 30 September 1987 see the whole document, especially page 31	1-14
A	---	1-14
	Clinical Chemistry, volume 34, no. 9, September 1988, S.L. Morrison et al.: "Production and characterization of genetically engineered antibody molecules", pages 1668-1675 see the whole document	
	---	./.
<p>* Special categories of cited documents: **</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
5th February 1991	27 FEB 1991	
International Searching Authority	Signature of Authorised Officer	
EUROPEAN PATENT OFFICE	MISS T. FAZELAAR	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Bio Essays, volume 8, no. 2, February/ March 1988, M. Verhoeven et al.: "Engineering of antibodies", pages 74-78 see the whole document -----	1-14



# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9001964  
SA 41668

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on 22/02/91  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A- 2189506	28-10-87	AU-A- 7196387	29-10-87
		BE-A- 1001517	21-11-89
		EP-A- 0251440	07-01-88
		FR-A- 2601963	29-01-88
		JP-A- 63044881	25-02-88
		LU-A- 86855	07-12-87
<hr/>			
EP-A- 0239400	30-09-87	GB-A, B 2188638	07-10-87
		JP-A- 62296890	24-12-87
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